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NEWS	2		"Ask CAS" for self-help around the clock
NEWS	3	SEP 01	New pricing for the Save Answers for SciFinder Wizard within STN Express with Discover!
NEWS	4	OCT 28	KOREAPAT now available on STN
NEWS	5	NOV 30	PHAR reloaded with additional data
NEWS	6	DEC 01	LISA now available on STN
NEWS	7	DEC 09	12 databases to be removed from STN on December 31, 2004
NEWS	8	DEC 15	MEDLINE update schedule for December 2004
NEWS	9	DEC 17	ELCOM reloaded; updating to resume; current-awareness alerts (SDIs) affected
NEWS	10	DEC 17	COMPUAB reloaded; updating to resume; current-awareness alerts (SDIs) affected
NEWS	11	DEC 17	SOLIDSTATE reloaded; updating to resume; current-awareness alerts (SDIs) affected
NEWS	12	DEC 17	CERAB reloaded; updating to resume; current-awareness alerts (SDIs) affected
NEWS	13	DEC 17	THREE NEW FIELDS ADDED TO IFIPAT/IFIUDB/IFICDB
NEWS	14	DEC 30	EPFULL: New patent full text database to be available on STN
NEWS	15	DEC 30	CAPLUS - PATENT COVERAGE EXPANDED
NEWS	16	JAN 03	No connect-hour charges in EPFULL during January and February 2005
NEWS	17	FEB 25	CA/CAPLUS - Russian Agency for Patents and Trademarks (ROSPATENT) added to list of core patent offices covered
NEWS	18	FEB 10	STN Patent Forums to be held in March 2005
NEWS	19	FEB 16	STN User Update to be held in conjunction with the 229th ACS National Meeting on March 13, 2005
NEWS	20	FEB 28	PATDPAFULL - New display fields provide for legal status data from INPADOC
NEWS	21	FEB 28	BABS - Current-awareness alerts (SDIs) available
NEWS	22	FEB 28	MEDLINE/LMEDLINE reloaded
NEWS	23	MAR 02	GBFULL: New full-text patent database on STN
NEWS	24	MAR 03	REGISTRY/ZREGISTRY - Sequence annotations enhanced
NEWS	25	MAR 03	MEDLINE file segment of TOXCENTER reloaded

NEWS EXPRESS	JANUARY 10 CURRENT WINDOWS VERSION IS V7.01a, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 10 JANUARY 2005
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FILE 'HOME' ENTERED AT 18:05:21 ON 11 MAR 2005

=> file biosis, medline, uspatful, biotechds
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FILE 'BIOSIS' ENTERED AT 18:08:17 ON 11 MAR 2005
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FILE 'USPATFULL' ENTERED AT 18:08:17 ON 11 MAR 2005
CA INDEXING COPYRIGHT (C) 2005 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOTECHDS' ENTERED AT 18:08:17 ON 11 MAR 2005
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=> s GFP and analog
L1 3776 GFP AND ANALOG

=> s l1 and mutant
L2 2882 L1 AND MUTANT

=> s l2 and (position F64 or S65 or E222 or S175)
'E222' NOT FOUND
The E# entered is not currently defined.

=> s l2 and (F64 or S65)
L3 73 L2 AND (F64 OR S65)

=> s l3 and (E222G)
L4 8 L3 AND (E222G)

=> s l4 and (S175)
L5 1 L4 AND (S175)

=> d l5 ti abs ibib tot

L5 ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
TI Novel fluorescent protein derived from green fluorescent protein useful as a transfection marker, has different excitation spectrum and/or emission spectrum compared with wild-type green fluorescent protein; recombinant green fluorescent protein production in transformed mammal, bacterium, yeast or insect cell culture
AN 2003-06533 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A fluorescent protein (I) derived from green fluorescent protein (**GFP**) or any functional **GFP analog**, has an amino acid sequence which is modified by amino acid substitution at position **F64**, at position **S65** or E222, and at position **S175** compared with the amino acid sequence of wild-type **GFP**, and has different excitation spectrum and/or emission spectrum compared with wild-type **GFP**, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a fusion compound (II) comprising a protein of interest fused to (I); (2) a nucleic acid molecule (III) comprising a nucleotide sequence encoding (I) or (II); (3) an expression vector (IV) comprising suitable expression control sequences operably linked to (III); and (4) a host cell (V) transformed or transfected with a DNA construct comprising (IV).

BIOTECHNOLOGY - Preparation: (I) is prepared by cultivating (V) and obtaining the polypeptide expressed by the nucleotide sequence (claimed). Preferred Protein: In (I), the amino acid F at position 64 is substituted

by an amino acid L, I, V, A or G. The amino acid S at position 175 is substituted by an amino acid G, A, L, I or T. The amino acid S at position 65 is substituted by an amino acid G, A, L, C, V, I or T. The amino acid E at position 222 is substituted by an amino acid G, A, V, L, I, F, S, T, N or Q. (I) is F64L-S175G-**E222G-GFP** or F64L-S65T-S175G-**GFP**. (I) has an amino acid sequence which is modified by amino acid substitution compared with wild-type **GFP** having a sequence of 238 amino acids fully defined in the specification. Preferred Host Cell: (V) is a mammalian cell, bacterial cell, yeast cell, or an insect cell.

USE - (III) is useful for measuring the expression of a protein of interest in a cell, by introducing (III) into a cell, where (III) is operably linked to and under the control of an expression control sequence which moderates expression of the protein of interest, culturing the cell under conditions suitable for the expression of the protein of interest, and detecting the fluorescence emission of **GFP** or functional **GFP analog**. (III) is useful for determining the cellular and/or extracellular localization of a protein of interest. (III) is also useful for comparing the effect of one or more test substance(s) on the expression and/or localization of one or more different protein(s) of interest in a cell. The method involves: (a) introducing into a cell, (III) operably linked to and under the control of a first expression control sequence and optionally fused to a nucleotide sequence encoding a fusion protein of interest, and optionally, at least one different nucleic acid molecule encoding a protein reporter molecule fused to a different protein of interest, where the nucleic acid molecule is operably linked to and under the control of a second expression control sequence, and the protein reporter molecule has or is capable of generating an emission signal which is spectrally distinct from that of **GFP** or functional **GFP analog**; (b) culturing the cells under conditions suitable for the expression of the protein(s) of interest in the presence and absence of the test substance(s); (c) determining the expression and/or localization of the protein(s) in the cells by detecting the fluorescence emission by optical means; and (d) comparing the fluorescence emission obtained in the presence and absence of the test substance(s). The samples of the cells in a fluid medium are introduced into separate vessels for each of the test substances to be studied (all claimed). (I) is useful as a non-toxic marker for selection of transfected cells, as a protein label in living and fixed cells, as a marker in cell or organelle fusion, for visualizing translocation of intracellular proteins to a specific organelle, as a secretion marker, as genetic reporter or protein tag for protein and gene expression in transgenic animals, as a cell or organelle integrity marker, as a transfection marker, as a marker to be used in combination with fluorescent activated cell sorting (FACS), as real-time probe working at near physiological concentrations, for performing transposon vector mutagenesis, and as a reporter for bacterial detection.

ADVANTAGE - (I) exhibits enhanced fluorescence relative to wild type **GFP**, when expressed in non-homologous cells at temperatures above 30degreesC, and excited at 490 nm. (I) detects **GFP** reporters in mammalian cells at lower levels of expression with increased sensitivity relative to wild type **GFP**.

EXAMPLE - Generation of mutants of green fluorescent protein (**GFP**) was as follows. The **GFP** gene was contained within the plasmid pGFP. The gene was amplified by polymerase chain reaction (PCR) using plaque forming units (pfu) polymerase. The primers were **GFP**-1: 5'-ggtacgggccgccaccatgagtaaaggagaagaactttcac, **GFP** -2: 5'-ggtacgggttaaccggttttgtatagttcatccatg, and **GFP**-3: 5'-ggtacgggccgccaccatgggatccaaaggagaagaacttttcac. Amplified products resulting from PCR reactions were tailed with a single 3'-deoxyadenosine using Taq polymerase and ligated into the TA cloning vector pTARGET. The mutants of **GFP** gene (encoding a sequence of 238 amino acids fully defined in the specification) construct such as F64L-S175G-**E222G-GFP** and F64L-S65T-**S175-GFP** within pTARGET were generated using the QuickChange site-directed mutagenesis kit. The primers used for F64L were **GFP**-64f: ccaacacttgtcactactctctcttatggtgttcaat and **GFP**-64r: attgaacaccataagagagagtagtgacaagtgttgg, S65T were **GFP**-65f:

ccaacacttgtcactactctcacctatggtgttcaatgcttttca and **GFP**-65r: tgaaaagcattgaacaccataggtgagagtagtgacaagtgttgg, S175G were **GFP** -175f: caacatgaagatggaggcgttcaactagcagacc and **GFP**-175r: ggtctgctagttgaacgcctccatcttcaatgttg, and **E222G** were (**GFP**-222f: ccacatggtccttcttggctttgtaacagctgctgg and **GFP** -222r: ccagcagctgttacaaagccaagaaggaccatgtgg). Multiply-mutated **GFP** molecules were generated through successive mutagenesis reactions. All **GFP mutant** sequences were verified by automated sequencing. The influence of individual mutations and combinations of F64L, S65T, V163A, S175G and **E222G** mutations upon **GFP** when expressed in mammalian cells was evaluated. Plasmid DNA to be used for transfection was prepared. DNA was diluted to 100 ng.microl-1 in 18-Megohm water and 1 microg used for transfections. For 50-80% confluency on the day of transfection, HeLa cells were plated at a density of 5x104/well in 6-well plates and incubated overnight. A 1:3 (1 microg:3 microl) ratio of DNA to FuGene6 reagent was used for each transient transfection reaction. 3microl FuGene 6 was added to 87 microl serum-free Dulbecco's modified Eagle medium (DMEM) and gently tapped to mix. Then 10 microl (1 microg) construct DNA was added and again gently mixed. The FuGene6:DNA complex was incubated at room temperature for 40 minutes, then added dropwise directly to the cells without changing the medium, and the plates swirled for even distribution. Fluorescence measurements were made 24 and 48 h after transfection. Average fluorescent intensities from fluorescent activated cell sorting (FACS) analysis were obtained. (52 pages)

ACCESSION NUMBER: 2003-06533 BIOTECHDS
TITLE: Novel fluorescent protein derived from green fluorescent protein useful as a transfection marker, has different excitation spectrum and/or emission spectrum compared with wild-type green fluorescent protein;
recombinant green fluorescent protein production in transformed mammal, bacterium, yeast or insect cell culture
AUTHOR: STUBBS S L J; JONES A E; MICHAEL N P; THOMAS N
PATENT ASSIGNEE: AMERSHAM PHARMACIA BIOTECH UK LTD; AMERSHAM BIOSCIENCES UK LTD
PATENT INFO: GB 2374868 30 Oct 2002
APPLICATION INFO: GB 2001-23288 28 Sep 2001
PRIORITY INFO: GB 2001-9858 23 Apr 2001; GB 2001-9858 23 Apr 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-095652 [09]

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(FILE 'HOME' ENTERED AT 18:05:21 ON 11 MAR 2005)

FILE 'BIOSIS, MEDLINE, USPATFULL, BIOTECHDS' ENTERED AT 18:08:17 ON 11 MAR 2005

L1 3776 S GFP AND ANALOG
L2 2882 S L1 AND MUTANT
L3 73 S L2 AND (F64 OR S65)
L4 8 S L3 AND (E222G)
L5 1 S L4 AND (S175)

=> d l4 ti abs ibib tot

L4 ANSWER 1 OF 8 USPATFULL on STN
TI Long wavelength engineered fluorescent proteins
AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2004:18826 USPATFULL
TITLE: Long wavelength engineered fluorescent proteins
INVENTOR(S): Wachter, Rebekka M., Creswell, OR, UNITED STATES
Remington, S. James, Eugene, OR, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2004014128	A1	20040122
APPLICATION INFO.:	US 2003-620099	A1	20030714 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2000-575847, filed on 19 May 2000, GRANTED, Pat. No. US 6593135 Continuation-in-part of Ser. No. US 1997-974737, filed on 19 Nov 1997, GRANTED, Pat. No. US 6077707 Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997, GRANTED, Pat. No. US 6054321 Continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996, GRANTED, Pat. No. US 6124128

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Lisa A. Haile, J.D., Ph.D., GRAY CARY WARE & FREIDENRICH LLP, Suite 1100, 4365 Executive Drive, San Diego, CA, 92121-2133
NUMBER OF CLAIMS: 187
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 62 Drawing Page(s)
LINE COUNT: 3919
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 2 OF 8 USPATFULL on STN
TI Long wavelength engineered fluorescent proteins
AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use are provided.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:51221 USPATFULL
TITLE: Long wavelength engineered fluorescent proteins
INVENTOR(S): Tsien, Roger Y., La Jolla, CA, UNITED STATES
Remington, James S., Eugene, OR, UNITED STATES
Cubitt, Andrew B., San Diego, CA, UNITED STATES
Heim, Roger, Del Mar, CA, UNITED STATES
Ormo, Mats F., Huddinge, SWEDEN
PATENT ASSIGNEE(S): The Regents of the University of California (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003036178	A1	20030220
	US 6780975	B2	20040824
APPLICATION INFO.:	US 2002-71976	A1	20020205 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1999-465142, filed on 16 Dec 1999, GRANTED, Pat. No. US 6403374 Continuation of Ser. No. US 1997-974737, filed on 19 Nov 1997, GRANTED, Pat. No. US 6077707 Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997, GRANTED, Pat. No. US 6054321 Continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996, GRANTED, Pat. No. US 6124128

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: GARY CARY WARE & FRIENDENRICH LLP, 4365 EXECUTIVE DRIVE, SUITE 1600, SAN DIEGO, CA, 92121-2189
NUMBER OF CLAIMS: 1
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 53 Drawing Page(s)

LINE COUNT: 2098
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 3 OF 8 USPATFULL on STN
TI LONG WAVELENGTH ENGINEERED FLUORESCENT PROTEINS
AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:17397 USPATFULL
TITLE: LONG WAVELENGTH ENGINEERED FLUORESCENT PROTEINS
INVENTOR(S): Wachter, Rebekka M., Creswell, OR, UNITED STATES
Remington, S. James, Eugene, OR, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003013149	A1	20030116
	US 6593135	B2	20030715
APPLICATION INFO.:	US 2000-575847	A1	20000519 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1997-974737, filed on 19 Nov 1997, GRANTED, Pat. No. US 6077707 Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997, GRANTED, Pat. No. US 6054321 Continuation of Ser. No. US 1996-706408, filed on 30 Aug 1996, GRANTED, Pat. No. US 6124128

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Lisa A Haile Ph D, Gray Cary Ware & Freidenrich LLP, 4365 Executive Drive, Suite 1100, San Diego, CA, 92121-2133
NUMBER OF CLAIMS: 187
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 63 Drawing Page(s)
LINE COUNT: 3752
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 4 OF 8 USPATFULL on STN
TI Long wavelength engineered fluorescent proteins
AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use are provided.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:136818 USPATFULL
TITLE: Long wavelength engineered fluorescent proteins
INVENTOR(S): Tsien, Roger Y., La Jolla, CA, United States
Remington, S. James, Eugene, OR, United States
Cubitt, Andrew B., San Diego, CA, United States
Heim, Roger, Del Mar, CA, United States
Ormo , Mats F., Huddinge, SWEDEN
PATENT ASSIGNEE(S): The Regents of the University of California, Oakland, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6403374	B1	20020611
APPLICATION INFO.:	US 1999-465142		19991216 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1997-974737, filed on 19 Nov 1997, now patented, Pat. No. US 6077707 Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997, now patented, Pat. No. US 6054321 Continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996, now patented, Pat. No. US 6124128

	NUMBER	DATE

PRIORITY INFORMATION:	US 1996-24050P 19960816 (60)
DOCUMENT TYPE:	Utility
FILE SEGMENT:	GRANTED
PRIMARY EXAMINER:	Nashed, Nashaat T.
LEGAL REPRESENTATIVE:	Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS:	23
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	55 Drawing Figure(s); 53 Drawing Page(s)
LINE COUNT:	2152

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 5 OF 8 USPATFULL on STN

TI Long wavelength engineered fluorescent proteins

AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2000:128162 USPATFULL
TITLE:	Long wavelength engineered fluorescent proteins
INVENTOR(S):	Tsien, Roger Y., La Jolla, CA, United States Cubitt, Andrew B., San Diego, CA, United States Heim, Roger, Del Mar, CA, United States Ormo, Mats F., Huddinge, Sweden Remington, S. James, Eugene, OR, United States
PATENT ASSIGNEE(S):	The Regents of the University of California, Oakland, CA, United States (U.S. corporation) Aurora Biosciences, La Jolla, CA, United States (U.S. corporation) The University of Oregon, Eugene, OR, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6124128		20000926	
APPLICATION INFO.:	US 1996-706408		19960830	(8)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	Granted
PRIMARY EXAMINER:	Achutamurthy, Ponnathapura
ASSISTANT EXAMINER:	Nashed, Nashaat T.
LEGAL REPRESENTATIVE:	Fish & Richardson P.C.
NUMBER OF CLAIMS:	37
EXEMPLARY CLAIM:	9
NUMBER OF DRAWINGS:	55 Drawing Figure(s); 53 Drawing Page(s)
LINE COUNT:	1735

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 6 OF 8 USPATFULL on STN

TI Long wavelength engineered fluorescent proteins

AB This invention provides functional engineered fluorescent proteins with varied fluorescence characteristics that can be easily distinguished from currently existing green and blue fluorescent proteins. In one aspect, the invention provides nucleic acids, expression vectors and recombinant host cells comprising nucleotide sequences encoding functional engineered fluorescent proteins comprising aromatic substitutions at position 66 and a folding mutation. In one embodiment the invention provides for fluorescent proteins containing an aromatic substitution at Thr 203.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2000:77223 USPATFULL
TITLE:	Long wavelength engineered fluorescent proteins
INVENTOR(S):	Tsien, Roger Y., La Jolla, CA, United States Remington, S. James, Eugene, OR, United States Cubitt, Andrew B., San Diego, CA, United States Heim, Roger, Del Mar, CA, United States Ormo, Mats F., Huddinge, Sweden
PATENT ASSIGNEE(S):	The Regents of the University of California, Oakland,

CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6077707		20000620
APPLICATION INFO.:	US 1997-974737		19971119 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997 which is a continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Nashed, Nashaat
LEGAL REPRESENTATIVE: Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 53 Drawing Figure(s); 53 Drawing Page(s)
LINE COUNT: 2162
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 7 OF 8 USPATFULL on STN
TI Long wavelength engineered fluorescent proteins
AB This invention provides functional engineered fluorescent proteins with varied fluorescence characteristics that can be easily distinguished from currently existing green and blue fluorescent proteins. In one embodiment the invention provides for the three dimensional structure and atomic coordinates of an Aequorea green fluorescent protein and methods for their use. In one embodiment, this invention provides a computational method of modeling the three dimensional structure of any other fluorescent protein based on the three dimensional structure of an Aequorea green fluorescent protein.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:50571 USPATFULL
TITLE: Long wavelength engineered fluorescent proteins
INVENTOR(S): Tsien, Roger Y., La Jolla, CA, United States
Remington, S. James, Eugene, OR, United States
Cubitt, Andrew B., San Diego, CA, United States
Heim, Roger, Del Mar, CA, United States
Ormo, Mats F., Huddinge, Sweden
PATENT ASSIGNEE(S): The Regents of the University of California, Oakland, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6054321		20000425
APPLICATION INFO.:	US 1997-911825		19970815 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Nashed, Nashaat
LEGAL REPRESENTATIVE: Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS: 15
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 36 Drawing Figure(s); 53 Drawing Page(s)
LINE COUNT: 2254
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 8 OF 8 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
TI Novel fluorescent protein derived from green fluorescent protein useful

as a transfection marker, has different excitation spectrum and/or emission spectrum compared with wild-type green fluorescent protein; recombinant green fluorescent protein production in transformed mammal, bacterium, yeast or insect cell culture

AN 2003-06533 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A fluorescent protein (I) derived from green fluorescent protein (**GFP**) or any functional **GFP analog**, has an amino acid sequence which is modified by amino acid substitution at position **F64**, at position **S65** or E222, and at position S175 compared with the amino acid sequence of wild-type **GFP**, and has different excitation spectrum and/or emission spectrum compared with wild-type **GFP**, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a fusion compound (II) comprising a protein of interest fused to (I); (2) a nucleic acid molecule (III) comprising a nucleotide sequence encoding (I) or (II); (3) an expression vector (IV) comprising suitable expression control sequences operably linked to (III); and (4) a host cell (V) transformed or transfected with a DNA construct comprising (IV).

BIOTECHNOLOGY - Preparation: (I) is prepared by cultivating (V) and obtaining the polypeptide expressed by the nucleotide sequence (claimed). Preferred Protein: In (I), the amino acid F at position 64 is substituted by an amino acid L, I, V, A or G. The amino acid S at position 175 is substituted by an amino acid G, A, L, I or T. The amino acid S at position 65 is substituted by an amino acid G, A, L, C, V, I or T. The amino acid E at position 222 is substituted by an amino acid G, A, V, L, I, F, S, T, N or Q. (I) is F64L-S175G-**E222G-GFP** or F64L-S65T-S175G-**GFP**. (I) has an amino acid sequence which is modified by amino acid substitution compared with wild-type **GFP** having a sequence of 238 amino acids fully defined in the specification. Preferred Host Cell: (V) is a mammalian cell, bacterial cell, yeast cell, or an insect cell.

USE - (III) is useful for measuring the expression of a protein of interest in a cell, by introducing (III) into a cell, where (III) is operably linked to and under the control of an expression control sequence which moderates expression of the protein of interest, culturing the cell under conditions suitable for the expression of the protein of interest, and detecting the fluorescence emission of **GFP** or functional **GFP analog**. (III) is useful for determining the cellular and/or extracellular localization of a protein of interest. (III) is also useful for comparing the effect of one or more test substance(s) on the expression and/or localization of one or more different protein(s) of interest in a cell. The method involves: (a) introducing into a cell, (III) operably linked to and under the control of a first expression control sequence and optionally fused to a nucleotide sequence encoding a fusion protein of interest, and optionally, at least one different nucleic acid molecule encoding a protein reporter molecule fused to a different protein of interest, where the nucleic acid molecule is operably linked to and under the control of a second expression control sequence, and the protein reporter molecule has or is capable of generating an emission signal which is spectrally distinct from that of **GFP** or functional **GFP analog**; (b) culturing the cells under conditions suitable for the expression of the protein(s) of interest in the presence and absence of the test substance(s); (c) determining the expression and/or localization of the protein(s) in the cells by detecting the fluorescence emission by optical means; and (d) comparing the fluorescence emission obtained in the presence and absence of the test substance(s). The samples of the cells in a fluid medium are introduced into separate vessels for each of the test substances to be studied (all claimed). (I) is useful as a non-toxic marker for selection of transfected cells, as a protein label in living and fixed cells, as a marker in cell or organelle fusion, for visualizing translocation of intracellular proteins to a specific organelle, as a secretion marker, as genetic reporter or protein tag for protein and gene expression in transgenic animals, as a cell or organelle integrity marker, as a transfection marker, as a marker to be used in combination with fluorescent activated cell sorting (FACS), as real-time

probe working at near physiological concentrations, for performing transposon vector mutagenesis, and as a reporter for bacterial detection.

ADVANTAGE - (I) exhibits enhanced fluorescence relative to wild type **GFP**, when expressed in non-homologous cells at temperatures above 30degreesC, and excited at 490 nm. (I) detects **GFP** reporters in mammalian cells at lower levels of expression with increased sensitivity relative to wild type **GFP**.

EXAMPLE - Generation of mutants of green fluorescent protein (**GFP**) was as follows. The **GFP** gene was contained within the plasmid pGFP. The gene was amplified by polymerase chain reaction (PCR) using plaque forming units (pfu) polymerase. The primers were **GFP**-1: 5'-ggtacgggccgccaccatgagtaaaggagaagaactttcac, **GFP** -2: 5'-ggtacgggttaaccggttttgtatagttcatccatg, and **GFP**-3: 5'-ggtacgggccgccaccatgggatccaaaggagaagaactttcac. Amplified products resulting from PCR reactions were tailed with a single 3'-deoxyadenosine using Taq polymerase and ligated into the TA cloning vector pTARGET. The mutants of **GFP** gene (encoding a sequence of 238 amino acids fully defined in the specification) construct such as F64L-S175G-**E222G-GFP** and F64L-S65T-S175-**GFP** within pTARGET were generated using the QuickChange site-directed mutagenesis kit. The primers used for F64L were **GFP**-64f: ccaacacttgtcactactctctcttatggtgttcaat and **GFP**-64r: attgaacaccataagagagagtagtgacaagtgttgg, S65T were **GFP**-65f: ccaacacttgtcactactctcacctatggtgttcaatgcttttca and **GFP**-65r: tgaaaagcattgaacaccataggtgagagtagtgacaagtgttgg, S175G were **GFP** -175f: caacatgaagatggaggcgttcaactagcagacc and **GFP**-175r: ggtctgctagttgaacgcctccatcttcaatgttg, and **E222G** were (**GFP**-222f: ccacatggtccttcttggctttgtaacagctgctgg and **GFP** -222r: ccagcagctgttacaaagccaagaaggaccatgtgg). Multiply-mutated **GFP** molecules were generated through successive mutagenesis reactions. All **GFP mutant** sequences were verified by automated sequencing. The influence of individual mutations and combinations of F64L, S65T, V163A, S175G and **E222G** mutations upon **GFP** when expressed in mammalian cells was evaluated. Plasmid DNA to be used for transfection was prepared. DNA was diluted to 100 ng.microl-1 in 18-Megohm water and 1 microg used for transfections. For 50-80% confluency on the day of transfection, HeLa cells were plated at a density of 5x104/well in 6-well plates and incubated overnight. A 1:3 (1 microg:3 microl) ratio of DNA to FuGene6 reagent was used for each transient transfection reaction. 3microl FuGene 6 was added to 87 microl serum-free Dulbecco's modified Eagle medium (DMEM) and gently tapped to mix. Then 10 microl (1 microg) construct DNA was added and again gently mixed. The FuGene6:DNA complex was incubated at room temperature for 40 minutes, then added dropwise directly to the cells without changing the medium, and the plates swirled for even distribution. Fluorescence measurements were made 24 and 48 h after transfection. Average fluorescent intensities from fluorescent activated cell sorting (FACS) analysis were obtained. (52 pages)

ACCESSION NUMBER: 2003-06533 BIOTECHDS
TITLE: Novel fluorescent protein derived from green fluorescent protein useful as a transfection marker, has different excitation spectrum and/or emission spectrum compared with wild-type green fluorescent protein;
recombinant green fluorescent protein production in transformed mammal, bacterium, yeast or insect cell culture
AUTHOR: STUBBS S L J; JONES A E; MICHAEL N P; THOMAS N
PATENT ASSIGNEE: AMERSHAM PHARMACIA BIOTECH UK LTD; AMERSHAM BIOSCIENCES UK LTD
PATENT INFO: GB 2374868 30 Oct 2002
APPLICATION INFO: GB 2001-23288 28 Sep 2001
PRIORITY INFO: GB 2001-9858 23 Apr 2001; GB 2001-9858 23 Apr 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-095652 [09]

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E7            6      STUBBUSCH JUTTA/AU
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E9            1      STUBBY J A/AU
E10           1      STUBBY JULIE/AU
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E12           1      STUBDAL A/AU

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E5            1      JONES3 S B/AU
E6            1      JONESA PETER G/AU
E7            1      JONESBRYANT NANCY/AU
E8            2      JONESCHEIT JENS/AU
E9            1      JONESCHILD DAVID E/AU
E10           1      JONESCHILD E S/AU
E11           3      JONESCHILD ELIZABETH/AU
E12           1      JONESCHILD ELIZABETH S/AU

=> e michael, N/au
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E2            1      MICHAEL ZINK/AU
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E4            1      MICHAELA A R/AU
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E12           2      MICHAELACCI L/AU
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☐ 1. Document ID: US 6780975 B2

L4: Entry 1 of 17 File: USPT Aug 24, 2004

US-PAT-NO: 6780975
DOCUMENT-IDENTIFIER: US 6780975 B2

TITLE: Long wavelength engineered fluorescent proteins

DATE-ISSUED: August 24, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Remington; S. James	Eugene	OR		
Cubitt; Andrew B.	San Diego	CA		
Heim; Roger	Del Mar	CA		
Ormo ; Mats F.	Huddinge			SE

US-CL-CURRENT: 530/350; 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

☐ 2. Document ID: US 6730520 B2

L4: Entry 2 of 17 File: USPT May 4, 2004

US-PAT-NO: 6730520
DOCUMENT-IDENTIFIER: US 6730520 B2

TITLE: Low fluorescence assay platforms and related methods for drug discovery

DATE-ISSUED: May 4, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Coassin; Peter J.	Encinitas	CA		
Harootunian; Alec Tate	Del Mar	CA		
Pham; Andrew A.	Del Mar	CA		
Stylli; Harry	San Diego	CA		
Tsien; Roger Y.	La Jolla	CA		

US-CL-CURRENT: 436/172; 422/102, 422/58, 422/61, 422/82.05, 422/82.08, 436/164, 436/165, 436/63, 436/71, 436/86

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

☐ 3. Document ID: US 6517781 B1

L4: Entry 3 of 17 File: USPT Feb 11, 2003

US-PAT-NO: 6517781
DOCUMENT-IDENTIFIER: US 6517781 B1

TITLE: Low fluorescence assay platforms and related methods for drug discovery

DATE-ISSUED: February 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Coassin; Peter J.	Encinitas	CA		
Harootunian; Alec Tate	Del Mar	CA		
Pham; Andrew A.	Del Mar	CA		
Stylli; Harry	San Diego	CA		
Tsien; Roger Y.	La Jolla	CA		

US-CL-CURRENT: 422/102; 422/100, 422/99, 435/283.1, 435/288.3, 435/288.4

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

☐ 4. Document ID: US 6403374 B1

L4: Entry 4 of 17 File: USPT Jun 11, 2002

US-PAT-NO: 6403374
DOCUMENT-IDENTIFIER: US 6403374 B1
** See image for Certificate of Correction **

TITLE: Long wavelength engineered fluorescent proteins

DATE-ISSUED: June 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Remington; S. James	Eugene	OR		
Cubitt; Andrew B.	San Diego	CA		
Heim; Roger	Del Mar	CA		
Ormo ; Mats F.	Huddinge			SE

US-CL-CURRENT: 435/325; 435/252.3, 435/252.33, 435/254.11, 435/320.1, 435/410, 536/23.1, 536/23.4, 536/23.6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

☐ 5. Document ID: US 6232114 B1

L4: Entry 5 of 17 File: USPT May 15, 2001

US-PAT-NO: 6232114
DOCUMENT-IDENTIFIER: US 6232114 B1

TITLE: Low background multi-well plates for fluorescence measurements of biological and biochemical samples

DATE-ISSUED: May 15, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Coassin; Peter J.	Encinitas	CA		
Harootunian; Alec Tate	Del Mar	CA		
Pham; Andrew A.	Del Mar	CA		
Tsien; Roger Y.	La Jolla	CA		

US-CL-CURRENT: 435/288.4; 422/102, 422/82.05

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw Desc | Ima

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☐ 6. Document ID: US 6229603 B1

L4: Entry 6 of 17 File: USPT May 8, 2001

US-PAT-NO: 6229603
DOCUMENT-IDENTIFIER: US 6229603 B1

TITLE: Low background multi-well plates with greater than 864 wells for spectroscopic measurements

DATE-ISSUED: May 8, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Coassin; Peter J.	Encinitas	CA		
Harootunian; Alec Tate	San Diego	CA		
Tsien; Roger Y.	La Jolla	CA		
Pham; Andrew A.	Del Mar	CA		

US-CL-CURRENT: 356/246; 356/440

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw Desc | Ima

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☐ 7. Document ID: US 6221612 B1

L4: Entry 7 of 17 File: USPT Apr 24, 2001

US-PAT-NO: 6221612
DOCUMENT-IDENTIFIER: US 6221612 B1

TITLE: Photon reducing agents for use in fluorescence assays

DATE-ISSUED: April 24, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Knapp; Tom	Encinitas	CA		
Zlokarnik; Gregor	San Diego	CA		
Negulescu; Paul	Del Mar	CA		
Tsien; Roger Y.	La Jolla	CA		
Rink; Tim	Monaco			MC

US-CL-CURRENT: 435/7.1; 422/82.05, 422/99, 427/102, 427/157, 427/213.34, 435/230, 435/5, 435/6, 435/7.2, 435/7.21, 435/7.5, 435/91.1, 436/501, 436/528, 436/529, 436/530, 436/531, 436/546, 436/800, 436/809

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

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☐ 8. Document ID: US 6214563 B1

L4: Entry 8 of 17 File: USPT Apr 10, 2001

US-PAT-NO: 6214563
DOCUMENT-IDENTIFIER: US 6214563 B1

TITLE: Photon reducing agents for reducing undesired light emission in assays

DATE-ISSUED: April 10, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Negulescu; Paul	Del Mar	CA		
Zlokarnik; Gregor	San Diego	CA		
Knapp; Tom	Encinitas	CA		
Tsien; Roger Y.	La Jolla	CA		
Rink; Tim	La Jolla	CA		

US-CL-CURRENT: 435/7.1; 422/82.05, 422/99, 427/102, 427/157, 427/213.34, 435/230, 435/5, 435/6, 435/7.2, 435/7.72, 435/7.92, 435/91.1, 436/501, 436/528, 436/529, 436/530, 436/531, 436/546, 436/800, 436/809

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

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☐ 9. Document ID: US 6200762 B1

L4: Entry 9 of 17 File: USPT Mar 13, 2001

US-PAT-NO: 6200762
DOCUMENT-IDENTIFIER: US 6200762 B1

TITLE: Photon reducing agents and compositions for fluorescence assays

DATE-ISSUED: March 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zlokarnik; Gregor	San Diego	CA		

Negulescu; Paul	Solana Beach	CA
Knapp; Tom	Encinitas	CA
Tsien; Roger Y.	La Jolla	CA
Rink; Tim	La Jolla	CA

US-CL-CURRENT: 435/7.1; 422/82.05, 422/99, 427/102, 427/157, 427/213.34, 435/230, 435/235.1, 435/5, 435/6, 435/7.2, 435/7.21, 435/7.24, 435/7.5, 435/7.72, 435/91.1, 436/501, 436/528, 436/529, 436/530, 436/531, 436/546, 436/800, 436/809

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

☐ 10. Document ID: US 6171780 B1

L4: Entry 10 of 17 File: USPT Jan 9, 2001

US-PAT-NO: 6171780
DOCUMENT-IDENTIFIER: US 6171780 B1

TITLE: Low fluorescence assay platforms and related methods for drug discovery

DATE-ISSUED: January 9, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Pham; Andrew A.	Del Mar	CA		
Coassin; Peter J.	Encinitas	CA		
Harootunian; Alec Tate	Del Mar	CA		
Stylli; Harry	San Diego	CA		
Tsien; Roger Y.	La Jolla	CA		

US-CL-CURRENT: 435/4; 422/102, 435/968, 435/975

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

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Terms	Documents
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L4	L3 and (F64 or E222 or (jp-07227287-$.did.) or (probe and surface adj enhanced adj raman adj scattering adjspectroscopy and metallic surface and metallic coating))	17	L4
L3	L2 and l1	64	L3
L2	GFP mutant or analog	345494	L2
L1	Tsien.in.	127	L1

END OF SEARCH HISTORY

easily distinguished from currently existing green and blue fluorescent proteins. Such engineered fluorescent proteins enable the simultaneous measurement of two or more processes within cells and can be used as fluorescence energy donors or acceptors when used to monitor protein-protein interactions through FRET. Longer wavelength engineered fluorescent proteins are particularly useful because photodynamic toxicity and auto-fluorescence of cells are significantly reduced at longer wavelengths. In particular, the introduction of the substitution T203X, wherein X is an aromatic amino acid, results in an increase in the excitation and emission wavelength maxima of Aequorea-related fluorescent proteins.

In one aspect, this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution located no more than about 0.5 nm from the chromophore of the engineered fluorescent protein, wherein the substitution alters the electronic environment of the chromophore, whereby the functional engineered fluorescent protein has a different fluorescent property than Aequorea green fluorescent protein.

In one aspect this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at T203 and, in particular, T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than Aequorea green fluorescent protein. In one embodiment, the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I. In another embodiment, the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S65G/V68L/Q69K/S72A/T203Y; S72A/S65G/V68L/T 203Y; S65G/S72A/T203Y; or S65G/S72A/T203W. In another embodiment, the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W. In another embodiment, the amino acid sequence farther comprises a mutation from Table A. In another embodiment, the amino acid sequence further comprises a folding mutation. In another embodiment, the nucleotide sequence encoding the protein differs from the nucleotide sequence of SEQ ID NO:1 by the substitution of at least one codon by a preferred mammalian codon. In another embodiment, the nucleic acid molecule encodes a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

In another aspect, this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than Aequorea green fluorescent protein. In one embodiment, amino acid substitution is:

L42X, wherein X is selected from C, F, H, W and Y,

V61X, wherein X is selected from F, Y, H and C,

T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C,

V68X, wherein X is selected from F, Y and H,

Q69X, wherein X is selected from K, R, E and G,

Q94X, wherein X is selected from D, E, H, K and N,

N121X, wherein X is selected from F, H, W and Y,

Y145X, wherein X is selected from W, C, F, L, E, H, K and Q,

H148X, wherein X is selected from F, Y, N, K, Q and R,

V150X, wherein X is selected from F, Y and H,

F165X, wherein X is selected from H, Q, W and Y,

I167X, wherein X is selected from F, Y and H,

Q183X, wherein X is selected from H, Y, E and K,

N185X, wherein X is selected from D, E, L, K and Q,

L220X, wherein X is selected from H, N, Q and T,

E222X, wherein X is selected from N and Q, or

V224X, wherein X is selected from H, N, Q, T, F, W and Y.

In a further aspect, this invention provides an expression vector comprising expression control sequences operatively linked to any of the aforementioned nucleic acid molecules. In a further aspect, this invention provides a recombinant host cell comprising the aforementioned expression vector.

In another aspect, this invention provides a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution located no more than about 0.5 cm from the chromophore of the engineered fluorescent protein, wherein the substitution alters the electronic environment of the chromophore, whereby the functional engineered fluorescent protein has a different fluorescent property than Aequorea green fluorescent protein.

In another aspect, this invention provides a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the amino acid substitution at T203, and in particular, T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than Aequorea green fluorescent protein. In one embodiment, the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I. In another embodiment, the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W. In another embodiment, the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W. In another embodiment, the amino acid sequence further comprises a folding mutation. In another embodiment, the engineered fluorescent protein is part of a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

In another aspect this invention provides a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of

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L7	6780975.pn.	1	L7
L6	l3 and (F64L/S175G/E222G)	0	L6
L5	L2 and (F64 or E222 or (jp-07227287-$.did.) or (probe and surface adj enhanced adj raman adj scattering adjspectroscopy and metallic surface and metallic coating))	48624	L5
L4	L3 and (F64 or E222 or (jp-07227287-$.did.) or (probe and surface adj enhanced adj raman adj scattering adjspectroscopy and metallic surface and metallic coating))	17	L4
L3	L2 and l1	64	L3
L2	GFP mutant or analog	345494	L2
L1	Tsien.in.	127	L1

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L1	6194548.pn.	1	L1

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☐ 1. Document ID: US 6852849 B2

L6: Entry 1 of 75 File: USPT Feb 8, 2005

US-PAT-NO: 6852849
DOCUMENT-IDENTIFIER: US 6852849 B2

TITLE: Non-oligomerizing tandem fluorescent proteins

DATE-ISSUED: February 8, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Campbell; Robert E.	San Diego	CA		

US-CL-CURRENT: 536/23.7; 435/320.1, 435/325, 435/69.1, 435/69.7, 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw Desc | Ima

☐ 2. Document ID: US 6803188 B1

L6: Entry 2 of 75 File: USPT Oct 12, 2004

US-PAT-NO: 6803188
DOCUMENT-IDENTIFIER: US 6803188 B1

TITLE: Tandem fluorescent protein constructs

DATE-ISSUED: October 12, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Heim; Roger	Del Mar	CA		

US-CL-CURRENT: 435/6; 435/183, 435/212, 435/252.3, 435/320.1, 435/325, 435/69.1, 435/69.7, 435/7.2, 435/7.4, 435/7.71, 435/7.72, 530/350, 530/402, 536/23.1, 536/23.4, 536/24.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw Desc | Ima

☐ 3. Document ID: US 6800733 B2

L6: Entry 3 of 75 File: USPT Oct 5, 2004

US-PAT-NO: 6800733
DOCUMENT-IDENTIFIER: US 6800733 B2

TITLE: Modified green fluorescent proteins

DATE-ISSUED: October 5, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Heim; Roger	Del Mar	CA		

US-CL-CURRENT: 530/350; 530/855, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw Desc | Ima

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☐ 4. Document ID: US 6780975 B2

L6: Entry 4 of 75 File: USPT Aug 24, 2004

US-PAT-NO: 6780975
DOCUMENT-IDENTIFIER: US 6780975 B2

TITLE: Long wavelength engineered fluorescent proteins

DATE-ISSUED: August 24, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Remington; S. James	Eugene	OR		
Cubitt; Andrew B.	San Diego	CA		
Heim; Roger	Del Mar	CA		
Ormo ; Mats F.	Huddinge			SE

US-CL-CURRENT: 530/350; 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw Desc | Ima

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☐ 5. Document ID: US 6748345 B2

L6: Entry 5 of 75 File: USPT Jun 8, 2004

US-PAT-NO: 6748345
DOCUMENT-IDENTIFIER: US 6748345 B2

TITLE: Method of analyzing crystalline texture

DATE-ISSUED: June 8, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chou; Cheng Tsien	Oxford			GB

Dicks; Keith Graham	Buckinghamshire	GB
Rolland; Pierre	Les Ulis	FR

US-CL-CURRENT: 702/27; 378/70, 378/71, 378/73, 702/23

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

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☐ 6. Document ID: US 6730520 B2

L6: Entry 6 of 75 File: USPT May 4, 2004

US-PAT-NO: 6730520
DOCUMENT-IDENTIFIER: US 6730520 B2

TITLE: Low fluorescence assay platforms and related methods for drug discovery

DATE-ISSUED: May 4, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Coassin; Peter J.	Encinitas	CA		
Harootunian; Alec Tate	Del Mar	CA		
Pham; Andrew A.	Del Mar	CA		
Stylli; Harry	San Diego	CA		
Tsien; Roger Y.	La Jolla	CA		

US-CL-CURRENT: 436/172; 422/102, 422/58, 422/61, 422/82.05, 422/82.08, 436/164, 436/165, 436/63, 436/71, 436/86

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

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☐ 7. Document ID: US 6699687 B1

L6: Entry 7 of 75 File: USPT Mar 2, 2004

US-PAT-NO: 6699687
DOCUMENT-IDENTIFIER: US 6699687 B1

TITLE: Circularly permuted fluorescent protein indicators

DATE-ISSUED: March 2, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Baird; Geoffrey	Solana Beach	CA		

US-CL-CURRENT: 435/69.1; 435/320.1, 435/325, 530/350, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

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☐ 8. Document ID: US 6686458 B2

L6: Entry 8 of 75 File: USPT Feb 3, 2004

US-PAT-NO: 6686458
DOCUMENT-IDENTIFIER: US 6686458 B2

TITLE: Synthetic molecules that specifically react with target sequences

DATE-ISSUED: February 3, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Griffin; B. Albert	San Diego	CA		

US-CL-CURRENT: 536/23.1; 424/9.1, 424/9.36, 424/9.361, 424/9.42, 424/9.44, 424/9.6, 534/10, 534/16, 544/226, 544/4, 544/64, 546/3, 549/207, 549/3, 549/39, 556/30, 556/68, 556/70, 556/71, 556/72

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

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☐ 9. Document ID: US 6627449 B1

L6: Entry 9 of 75 File: USPT Sep 30, 2003

US-PAT-NO: 6627449
DOCUMENT-IDENTIFIER: US 6627449 B1

TITLE: Fluorescent protein sensors for measuring the pH of a biological sample

DATE-ISSUED: September 30, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Miyawaki; Atsushi	San Diego	CA		
Llopis; Juan	San Diego	CA		

US-CL-CURRENT: 436/86; 435/252.3, 435/254.11, 435/320.1, 435/325, 435/410, 435/810, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

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☐ 10. Document ID: US 6608671 B2

L6: Entry 10 of 75 File: USPT Aug 19, 2003

US-PAT-NO: 6608671
DOCUMENT-IDENTIFIER: US 6608671 B2

TITLE: Detector and screening device for ion channels

DATE-ISSUED: August 19, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Coassin; Peter J.	Encinitas	CA		
Pham; Andrew A.	Del Mar	CA		
Harootunian; Alec Tate	Del Mar	CA		
Vuong; Minh	San Diego	CA		

US-CL-CURRENT: 356/72; 356/436, 356/440, 422/82.08

Terms	Documents
L5 and (position (probe and surface adj enhanced adj raman adj scattering adjspectroscopy and metallic surface and metallic coating))	75